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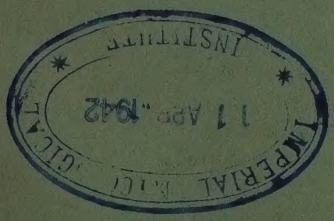

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THE BOTANICAL REVIEW

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POSSIBILITIES IN PLANT VIRUS CLASSIFICATION

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In respect to classification, the virus diseases of plants stand in sharp contrast to diseases caused by fungi. The latter are grouped in elaborate classifications based for the most part on the natural relationships of their etiological agents. Such a classification of virus diseases is not feasible at the present time, because little is known of the agents causing these diseases. A satisfactory classification of viruses may not be possible until it is proved whether they represent a group of minute living organisms or a series of non-living substances, and a more suitable technique than is now available for their study has been devised. While our present knowledge is too meager to permit the arrangement of plant viruses into groups on a well-founded basis of natural relationships, recent studies justify the hope that a fairly useful classification may soon be possible. Several methods of identifying and grouping viruses have been suggested, but all are somewhat limited in applicability. This paper will attempt to discuss these methods and the bearing of recent work on problems of classification.

SOME METHODS OF DIFFERENTIATING AND CLASSIFYING VIRUSES

Johnson (27, 28) has recommended a method employing physical and chemical properties, such as thermal death point, retention of activity *in vitro,* and reaction to chemicals, along with symptom manifestations in different host plants. Quanjer (56) has emphasized the value of histological and cytological symptoms for the identification and classification of potato viruses. Elze (19) and Storey (76) have suggested a classification of viruses by means of their insect relationships. Elze separates all viruses into two groups on the basis of whether or not they are transmitted by

insects. Those transmitted by insects are further separated on the basis of whether they are transmitted by one, or more than one, insect. The viruses transmitted by one insect are still further separated into groups having long and short incubation periods in their vectors. Such a classification seems of doubtful value, since it is generally believed that most, if not all, plant viruses are transmitted by insects. Those not now known to be so transmitted do not form a natural group. A division on the basis of transmission by one or by more than one insect also seems to separate viruses that are closely related. Most viruses that are spread by several insects are infectious enough to be transmitted mechanically. Some, however, such as that of potato leaf roll (19, 65, 87, 88), Fiji disease of sugar-cane (49, 50), and curly top of sugar beets (2, 20), seem to enter into a specific relationship with more than one insect. Far too little is known regarding incubation periods of viruses in insects to permit a classification on this basis at the present time.

Storey (76) suggests that in manner of transmission may be found an important criterion for the differentiation of viruses. He regards a specific insect vector of a virus as no less infected than the plant host and notes a remarkably close argreement between a grouping of viruses on the basis of symptom manifestation and a grouping according to the type of insect vector. Storey points out that mosaics are generally transmitted by aphids and yellows diseases by jassids. However, there are some mosaic diseases, such as the yellow bean mosaic of Haiti transmitted by *Empoasca fabalis* De Long (69), and the rice mosaic of Japan transmitted by *Nephotettix apicalis* Motsch (83), that are spread by jassids, and some diseases apparently of the yellows type, such as leaf roll of potato transmitted by *Myzus persicae* Sulz. (65), and the yellow-flat disease of lilies transmitted by *Aphis gossypii* Glover (23), that are spread by aphids. In spite of certain limitations of the method, Storey is undoubtedly correct in his views on the importance of insect relationships as useful criteria for the differentiation and classification of viruses.

IMPORTANCE OF BIOLOGICAL CARRIERS

The mechanical spread of viruses by insects is of little or no significance from the standpoint of natural relationships. A

highly infectious virus is likely to be spread by any insect that feeds on infected plants. Some fifty different species, including both aphids and jassids, are reported to spread the mosaic of onions (17) which is readily transmitted mechanically. This knowledge is of little value from the standpoint of virus classification. It is information regarding biological relationships that is useful for this purpose. Whether such relationships consist of infection of insects by the viruses they transmit, as is believed by Storey (76), or in an association of some other nature need not be discussed here. That an intimate specific relationship does exist is generally agreed. Evidence supporting this view is found in studies on insect spread of viruses not mechanically transmissible, on long retention of viruses by certain vectors, on incubation periods of viruses in vectors, and on other insect relationships.

A considerable number of virus diseases not known to be sap-transmissible are spread by insects. Aster yellows, carried by the leafhopper *Cicadula sexnotata* Fallen (35), is a good example of such a disease. In spite of many efforts to transfer the disease by rubbing or injecting sap from diseased plants into healthy plants, it has never been transmitted mechanically except by grafting. The vector, however, transmits the disease very readily. This malady also furnishes a good example of the retention of a virus by a vector. Newly hatched nymphs of *Cicadula sexnotata,* if allowed to become infective by feeding on yellowed aster plants for a few hours, usually retain the yellows virus throughout life even when cultured on rye or other plants immune to yellows. In a few instances it has been proved that viruses are transmitted by vectors only after an incubation period. The insects can not infect healthy plants immediately after first feeding on diseased plants. A definite period of time must elapse between the hour in which they first pick up virus and the hour in which they are first capable of transmitting it. The virus of curly top of sugar beets requires a minimum incubation period of from 4 to 48 hours in the leafhopper *Eutettix tenellus* Baker (63, 70), depending on the temperature at which the insect is held. The period is shortened by high temperatures. The viruses of potato leaf roll (18, 64, 65), streak disease of corn (73), bunchy top of abaca (51), and mosaic of peas (53) have incubation periods of similar lengths in their insect vectors. Longer incubation periods have

been reported for the virus of aster yellows in *Cicadula sexnotata* (34, 35), the virus of yellow spot of pineapple in *Thrips tabaci* Lind. (43, 44), and the virus of spotted wilt of tomato in *Frankliniella insularis* Frankl. (1) and *Thrips tabaci* (67). The aster-yellows and yellow-spot viruses require periods of about 10 days, while the spotted-wilt virus requires from 5 to 7 days. The incubation periods suggest that these viruses may pass through some stage of development, or at least may multiply, in their vectors.

Equally good evidence of a biological relationship is brought by studies on the relation between age of vectors and their ability to become infective. Adults of the species *Frankliniella insularis* (61) are unable to transmit the virus of spotted wilt unless they are hatched from infected pupae. Both larvae and adults can transmit the virus, but only the larvae can pick it up. A similar relationship exists between this virus and its other vector, *Thrips tabaci* (67), and between the yellow-spot virus of pineapples, which may or may not be identical with spotted-wilt virus, and *Thrips tabaci* (43). A somewhat similar relationship is suggested by Rankin's (58) report that very young instars of the first, second, and later generations of *Amphorophora rubi* Kalt. taken from mosaic-diseased raspberry leaves were infective, while their mothers and more mature sisters taken from the same leaves were noninfective. Fukushi's (21) discovery that rice-mosaic virus is transmitted through the eggs of its leafhopper vector, and Storey's (78) discovery that in the leafhopper species *Cicadulina mbila* Naude ability to transmit streak of corn is inherited as a simple dominant sex-linked Mendelian factor, and that individuals lacking this factor may become infective by needle inoculations (80) although they do not become infective by feeding on diseased plants, bring further evidence of the delicate biological relationships that exist between insects and viruses.

It is possible, and even probable, that the highly infectious viruses, as well as those not readily sap-transmissible, are biologically carried by one or more insects. Evidence of this is, however, difficult to obtain because in such cases there is no easy way of separating the mechanical carriers from the biological carriers. Viruses that are not mechanically transmitted, or at least are not easily so transmitted, are spread by biological carriers only. In

such instances the biological relationship is not obscured by mechanical transmissions. Up to the present time no highly infectious virus has been shown to be biologically transmitted and proof of such transmission has been given for less than one fourth of the known plant viruses. When biological carrier relationships are better known and have been established for a large number of virus diseases, they may furnish a good basis for differentiation and classification.

Some examples of virus diseases believed to be biologically carried, together with their vectors, are listed below:

Curly top of sugar beets in the United States by *Eutettix tenellus* Baker (70).
Curly top of sugar beets in Argentina by *Agallia sticticollis* Stål. (20).
Mosaic of rice in Japan by *Nephotettix apicalis* Motsch (83).
Yellows of the China aster in the United States by *Cicadula sexnotata* Fallen (35).
Streak of corn in South Africa by *Cicadulina mbila* Naude (72).
Streak of corn in Tanganyika by *Cicadulina zeae* China (76).
Yellow dwarf of potato in the United States by *Agallia sanguinolenta* Prov. (7).
Fiji disease of sugar cane in Queensland, Australia, by *Perkinsiella saccharicida* Kirk. (49).
Fiji disease of sugar cane in the Philippine Islands by *Perkinsiella vastatrix* Bred. (50).
Yellow bean mosaic in Haiti by *Empoasca fabalis* De Long (69).
False blossom of cranberries in the United States by *Euscelis striatulus* Fall. (15, 16).
Peach yellows in the United States by *Macropsis trimaculata* Fitch (38).
Corn mosaic in Hawaii by *Peregrinus maidis* Ashm. (33).
Potato leaf roll in many different countries by *Myzus persicae* Sulz. (62) and by *Myzus circumflex* Buckton (87).
Sugar cane mosaic in many countries by *Aphis maidis* Fitch (10).
Red raspberry mosaic in the United States by *Amphorophora rubi* Kalt. (58) and *Aphis rubiphila* Patch (57).
Mosaic of peas and other leguminous plants in the United States by *Macrosiphum pisi* Kalt. and *Macrosiphum gei* Koch (53).
Peanut rosette in Africa by *Aphis leguminosae* Theobald (74).
Lily mosaic in the United States and other countries by *Aphis gossypii* Glover (23).
Yellow flat disease of lilies in Bermuda by *Aphis gossypii* Glover (52).
Banana bunchy top disease in Australia by *Pentalonia nigronervosa* Coq. (45).
Bunchy top of abaca in the Philippine Islands by *Pentalonia nigronervosa* Coq. (51).

Spotted wilt of tomato in Australia by *Frankliniella insularis* Frankl. (60).
Spotted wilt of tomato in England by *Thrips tabaci* Lind. (66).
Pineapple yellow spot in Hawaii by *Thrips tabaci* Lind. (42).
Leaf curl of cotton in the Sudan by *Bemisia gossypiperda* Misra and Lamba (31).
Leaf curl of tobacco in Rhodesia and elsewhere by *Bemisia gossypiperda* Misra and Lamba (77, 79).

The above list of biological carriers includes 13 jassids, 10 aphids, 2 thrips, and 1 whitefly. Most of the diseases in this list are known to be transmitted by one insect only and are undoubtedly caused by different virus entities. Several of the diseases are, however, transmitted by more than one insect, as was mentioned previously. The curly top of sugar beets in the United States is spread by the leafhopper *Eutettix tenellus* (2) only, but the curly top of sugar beets in Argentina, where *Eutettix tenellus* does not occur, is transmitted by another leafhopper, *Agallia sticticollis* (20). Whether or not the disease in Argentina is due to the same virus that causes curly top in the United States is not known. It seems identical from the standpoint of symptomatology. The Fiji disease of sugar cane is transmitted by *Perkinsiella saccharicida* in Queensland, Australia, (49), and by *P. vastatrix* in the Philippine Islands (50). Since the symptoms of this disease are distinct from those of all other known plant diseases, there can be little doubt that the virus in Australia is identical, or very closely related, to that prevalent in the Philippine Islands. If they are identical, we have here an excellent example of a virus that it biologically transmitted by two different, though closely related, insect species. A similar case is reported by Storey (76) for the streak disease of corn which is transmitted by both *Cicadulina mbila* and *C. zeae* in Tanganyika where the two species exist side by side. Storey proved that the virus in a plant infected by means of *C. mbila* may be taken up and transmitted by *C. zeae*. Osborn (53) has shown that pea mosaic, although not transmitted by *Aphis rumicis* L., is carried by both *Macrosiphum pisi* and *M. gei.* The spotted wilt of tomato, transmitted by *Frankliniella insularis* (60) and *Thrips tabaci* (66), is another good example of a virus that is biologically carried by two different insects. The leaf-roll disease of potato is reported to be spread by the following aphids:

Myzus persicae, Aphis rhamni Boyer (19), *Macrosiphum pelargonii* Kalt. (88), *M. gei* (87), *Myzus pseudosolani* Theobald (65), and *M. circumflexus* (87). Since the virus of this disease is not mechanically transmissible, it is assumed that a biological relationship exists between it and each of these aphid vectors. There are, on the other hand, a few instances of the biological transmission of two very different viruses by the same insect. *Aphis gossypii* transmits both the mosaic (23) and the yellow-flat (52) diseases of lily. These diseases are believed to be distinct. *Myzus persicae* is a vector of dahlia mosaic (14) and potato leaf roll (62), two very distinct diseases. *Pentalonia nigronervosa* transmits bunchy top of abaca in the Philippine Islands (51) and bunchy top of banana in Australia (45). These two diseases are very much alike, but the Philippine virus is not transmissible to bananas, either in the field or experimentally, by the vector which readily transmits the bunchy top virus to bananas in Australia. A similar instance is reported for *Cicadula sexnotata* which transmits both the eastern and the western types of aster yellows (37) in the United States. The two diseases are indistinguishable on asters, but the western or California type of yellows is readily transmitted to celery and Zinnia while the eastern type of yellows does not go to these plants.

It is evident from the above discussion that some of the viruses which are known to be biologically carried are not readily differentiated on the basis of vector relationships alone. Although such relationships are highly important, they are usually not sufficient for purposes of classification unless considered in connection with other characteristics of the viruses and the diseases they produce. This examination of some of the methods recommended for the classification of plant viruses leads to the conclusion that, while no one method is now applicable to all viruses, each method can be used to advantage with certain of them. As has already been stated by Quanjer (56) and Smith (68), much more information must be collected before a satisfactory and stable classification will be possible. It is, however, not to be expected that efforts in this direction will await completion of all the studies that may be necessary for a final classification. The arrangement of related viruses into groups is being accomplished gradually as new information

becomes available and new methods for virus differentiation are devised.

EVIDENCE OF RELATIONSHIPS AMONG THE PLANT VIRUSES

When a virus is found that affects some plant not previously known to be attacked by other viruses, or that produces in plants susceptible to other viruses symptoms distinguishable from those characteristic of diseases caused by known viruses, it is usually described as new and the disease it produces as a new disease without any attempt to relate it to other members of the virus group. This practice has led to the recognition and naming of many diseases caused by closely related viruses. Recent studies indicate that there are prevalent in nature a considerable number of viruses that are only slightly different from other viruses and may therefore be considered strains of the latter. In the opinion of the writer, these studies covering a wide range of diseases fully justify the conception of strains among the plant viruses.

Mention has already been made of the fact that a disease known as bunchy top affects bananas in Australia, and that a malady with similar symptoms affects abacas but not bananas in the Philippine Island, and of the eastern and western types of aster yellows in the United States that are identical as far as the vector and symptoms on aster plants are concerned but have slightly different host ranges. Two leaf-curl diseases (5) affecting red and black raspberries, and designated by Bennett as alpha curl and beta curl, are transmitted by *Aphis rubiphila* and are similar symptomatically except on the purple variety Columbian. One of them does not, however, attack the variety Black Cumberland. Storey (76) has demonstrated the existence of two strains of sugar cane mosaic that differ in host range. Both are transmitted by *Aphis maidis*.

Summers (82) reports the presence of four types of sugar cane mosaic in Louisiana. They are distinguished by symptom differences on different varieties of cane. Storey and McClean (75) found several different types of streak in East Africa. The virus of each disease shows a specialization for its own host. Thus the virus from corn streak produces a severe and permanent disease in corn, but only a transitory infection in Uba cane. The Uba cane streak virus produces a mild but permanent disease in cane. In corn it causes a very mild disease that tends to become sup-

pressed with the growth of the plant. The wild grass, *Digitaria horizontalis* Willd., is affected by a streak virus that differs from both the corn and the cane viruses. All of these viruses are spread by the same vector, *Cicadulina mbila.* A disease very similar to Storey's streak of corn is prevalent in the Hawaiian Islands. It was shown to be distinct from sugar cane mosaic (36) and to be transmitted from corn to corn, but not from corn to cane, by *Peregrinus maidis* (33). Stahl (71), working in Cuba, described a similar disease of corn which is also transmitted by *Peregrinus maidis. Cicadulina mbila* does not occur in Hawaii or Cuba, but *Peregrinus maidis* does occur in East Africa. It does not, however, transmit the East African streak of corn. These corn diseases of Hawaii, Cuba, and Africa are so similar in symptom manifestations that it is difficult to believe they are not closely related. The Hawaiian disease is readily diagnosed by the presence of characteristic inclusion bodies in affected cells of both stems and leaves (32). Since neither Stahl nor Storey has studied the stripe or streak diseases cytologically, nothing is known regarding them from the standpoint of cellular pathology. Conspicuous inclusion bodies should be found in cells of plants infected with stripe or streak if these diseases are related to the Hawaiian disease of corn. As was stated above, the Fiji disease of sugar cane in Australia is spread by the leafhopper *Perkinsiella saccharicida* (49). In the Philippine Islands where *P. saccharicida* does not occur, a similar, if not identical, disease of sugar cane is spread by *P. vastatrix* (50). Two types of kroepoek disease of tobacco are described by Kerling (29) and three types by Thung (85). All are transmitted by the same whitefly and are much alike except for severity of symptoms.

Carsner and Lackey have described attenuated strains of the sugar beet curly-top virus (11, 12, 40, 41). The strains were obtained by passage of ordinary curly-top virus through resistant varieties of sugar beets or through resistant weed species. Attenuated strains of tobacco mosaic obtained from infected plants or tissues after incubation at high temperatures have also been described (24, 26).

Böhme (8) reports the isolation of four types of X- (latent virus of potato) and three types of Y-virus (veinbanding mosaic

of potato) from potato. The types in both the X- and the Y-groups produce slightly different symptoms in certain host plants. One of the X-virus strains could not be mechanically transmitted to certain potato varieties except by grafting, while two of the others were transmissible by rubbing. One of the Y-virus strains was found to have a thermal death point of about 50° C., whereas another Y-virus strain was still infectious when heated to 55° C. The virus of yellow mosaic of tomato was found by Stover and Vermillion (81) to be inactivated at about 83° C., while that of ordinary tobacco mosaic was inactivated at about 90° C. The yellow mosaic is presumed to have been a strain of tobacco mosaic, since it caused streak on tomato when in combination with the latent virus of potato. Two strains of Abutilon mosaic differing in severity of symptoms are reported by Keur (30). Venkata Roa and Gopala Iyengar (86) have recently described two symptomatically distinct types of the spike disease of sandal.

That the different virus strains in the groups listed above may be related in the sense of one strain being derived from another is suggested by the fact that certain strains differ only in symptom intensity, in host range, in insect vector, in thermal death point, or in infectivity, but even better evidence of genetic relationships is now available.

McKinney (46) was the first to show that a yellow strain of tobacco mosaic may be obtained from the bright yellow spots that occasionally occur in the leaves of tobacco plants infected by the ordinary green tobacco mosaic. He suggested that the yellow strain may arise as a mutation from the green strain (47). McKinney (48) has likewise shown that a yellow strain of wheat mosaic may be isolated from wheat plants infected with the ordinary green-mosaic virus of wheat. Jensen (25) found that a whole series of yellow and necrotic strains of tobacco mosaic may be obtained from the bright yellow spots, and that the spots occur in the leaves of plants infected by ordinary tobacco-mosaic virus that has apparently been freed of any contaminating viruses by successive passage through primary necrotic lesions in leaves of *Nicotiana glutinosa* L. Some of the yellow strains isolated by Jensen appear to be identical with yellow strains of tobacco mosaic

occurring in nature. Others differ from any of the yellow strains that have been described previously. One of Jensen's strains causes symptoms similar to aucuba mosaic of tomato on tomato and tobacco plants and, like the latter, produces necrotic primary lesions on mature leaves of *N. sylvestris* Spegaz. and Comes and certain other host plants. Several of Jensen's strains are much less infectious than the virus of green tobacco mosaic from which they were derived.

Price (54) has isolated several different strains of yellow cucumber mosaic from bright yellow spots that occasionally occur in leaves of tobacco plants infected with ordinary cucumber-mosaic virus. All of these strains, as well as the ordinary green cucumber-mosaic virus, produce necrotic primary lesions in the leaves of cowpea plants of the variety Black Eye. None of them cause mottling in the cowpea. Among the necrotic lesions regularly produced on cowpea leaves by the green-mosaic virus, Price found two chlorotic lesions. From these he isolated a cucumber-mosaic virus that is systemic and causes mottling in cowpeas. He thus obtained experimentally a strain of cucumber-mosaic virus that differs from all other known strains in respect to symptoms on cowpeas. This strain differs symptomatically from the green cucumber-mosaic virus from which it was derived to about the same extent that the streak virus of corn in East Africa differs from Storey's streak virus of cane.

The work of McKinney, Jensen and Price proves that variant strains of both the tobacco- and the cucumber-mosaic viruses may be isolated from plants infected with viruses causing the ordinary types of these diseases, and, in addition, brings evidence that the variants are derived from the ordinary well known types. These results support the view that the variant strains isolated, as well as the several strains of both viruses, known to occur in nature, are genetically related. If this be true, the viruses causing diseases that differ only slightly from other diseases should be grouped together and treated as members of a naturally related family. We would thus assemble a tobacco-mosaic virus group, a cucumber-mosaic virus group, a corn-streak virus group, a sugar cane mosaic virus group, an X-virus (latent virus of potato) group, a Y-virus (potato veinbanding mosaic) group, etc.

NEW METHODS FOR DIFFERENTIATING PLANT VIRUSES

This brings us to a consideration of some further methods available for testing relationships suspected to exist between viruses causing diseases that resemble each other. Evidence indicating that certain plant viruses, or substances intimately associated with these viruses, are antigenic has been accumulating during recent years (3, 4, 6, 13, 22, 55). For example, there is now good reason to believe that the viruses of tobacco mosaic, cucumber mosaic, and tobacco ring-spot have this capacity. Each is readily distinguished from the other two antigenically. Chester (13) has recently shown that Jensen's tobacco-mosaic virus strains have similar antigenic properties, and that the different cucumber-mosaic virus strains isolated by Price are also alike in this respect. The strains in the tobacco group are, however, entirely distinct antigenically from strains in the cucumber-mosaic group, although certain members of the two groups show remarkably similar symptom manifestations. The results presented by Jensen and Price in support of the view that virus strains isolated from plants having tobacco mosaic or cucumber mosaic are related to tobacco-mosaic virus and cucumber-mosaic virus respectively, are thus confirmed. While the serological method may not prove suitable for the study of all plant viruses, it nevertheless offers interesting possibilities for virus classification.

A second method for testing virus relationships depends on the fact that the infection of a plant by one strain of a virus will, in some cases at least (9, 39, 59, 84), protect the plant against infection by a related strain, while it will give no protection against an unrelated strain. This method has been successfully used for the differentiation of strains of tobacco-mosaic virus from cucumber-mosaic virus and tobacco ring-spot virus (39). It will doubtless prove useful for the testing of relationships in other groups. The two methods are similar in that they depend on immunological reactions; the first on an immunological reaction in an animal, the second on an immunological reaction in a plant. Therefore, they offer the means for a more convenient and direct test of virus relationships than do some of the other criteria. It is believed that by using these two methods, in combination with other methods discussed, it may soon be possible to arrange most, if not all, of the

plant viruses into naturally related groups which may serve as a basis for the classification of virus diseases.

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Literature Cited

1. Bald, J. G., and Samuel, G. Investigations on spotted wilt of tomatoes. II. Bull. Counc. Sci. and Ind. Res. Austr. No. 54. 24 p. 1931.
2. Ball, E. D. The leafhoppers of the sugar beet and their relation to the "curly-leaf" condition. U. S. Dept. Agr. Bur. Ent. Bull. **66**: 35–52. 1909.
3. Beale, H. P. Specificity of the precipitin reaction in tobacco mosaic disease. Contr. Boyce Thompson Inst. **3**: 529–539. 1931.
4. Beale, H. P. The serum reactions as an aid in the study of filterable viruses of plants. Contr. Boyce Thompson Inst. **6**: 407–435. 1934.
5. Bennett, C. W. Further observations and experiments on the curl disease of raspberries. Phytopath. **20**: 787–802. 1930.
6. Birkeland, J. M. Serological studies of plant viruses. Bot. Gaz. **95**: 419–436. 1934.
7. Black, L. M. The potato yellow dwarf disease. Am. Potato Jour. **11**: 148–152. 1934.
8. Böhme, R. W. Vergleichende Untersuchungen mit Stämmen des "x" —und "y"—virus. Phytopath. Zeitschr. **6**: 517–524. 1933.
9. Botjes, J. G. O. Verzwakking van het virus der topnecrose, en verworven immuniteit van aardappelrassen ten opzichte van dit virus. Tijdschr. Plantenz. **39**: 249–262. 1933.
10. Brandes, E. W. Artificial and insect transmission of sugar cane mosaic. Jour. Agr. Res. **19**: 131–138. 1920.
11. Carsner, E. Attenuation of the virus of sugar beet curly-top. Phytopath. **15**: 745–757. 1925.
12. Carsner, E., and Lackey, C. F. Further studies on attenuation of the virus of sugar beet curly-top. Phytopath. **18**: 951. 1928.
13. Chester, K. S. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ringspot, and cucumber mosaic by immune sera. Phytopath. **24**: 1180–1202. 1934.
14. Brierley, P. Virus diseases of dahlia. Phytopath. **23**: 6. 1933.
15. Dobroscky, I. D. Cranberry false-blossom disease spread by a leafhopper. Science **70**: 635. 1929.
16. Dobroscky, I. D. Studies on cranberry false blossom disease and its insect vector. Contr. Boyce Thompson Inst. **3**: 59–83. 1931.
17. Drake, C. J., Tate, H. D., and Harris, H. M. The relationship of aphids to the transmission of yellow dwarf of onions. Jour. Econ. Ent. **26**: 841–846. 1933.

18. Elze, D. L. De verspreiding van virusziekten von de aardappel (*Solanum tuberosum* L.) door insekten. Inst. voor Phytopath. Lab. voor Mycol. en Aardappelonderzoek, Meded. **32**. 90 p. 1927.
19. Elze, D. L. The relation between insect and virus as shown in potato leaf roll and a classification of viroses based on this relation. Phytopath. **21**: 675–676. 1931.
20. Fawcett, G. L. The curly top of sugar beet in the Argentine. Phytopath. **17**: 407–408. 1927.
21. Fukushi, T. Transmission of the virus through the eggs of an insect vector. Proc. Imp. Acad. Tokyo **9**: 457–460. 1933.
22. Gratia, A. Pluralité antigénique et identification sérologique des virus des plantes. Compt. Rend. Soc. Biol. **114**: 923–924. 1933.
23. Guterman, C. E. F. Final summary of the work on diseases of lilies. Hort. Soc. N. Y. Yearbook **1930**: 51–102. 1930.
24. Holmes, F. O. A masked strain of tobacco-mosaic virus. Phytopath. **24**: 845–873. 1934.
25. Jensen, J. H. Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic. Phytopath. **23**: 964–974. 1933.
26. Johnson, J. The attenuation of plant viruses and the inactivating influence of oxygen. Science **64**: 845–873. 1934.
27. Johnson, J. The classification of plant viruses. Wisconsin Agr. Exp. Sta. Res. Bull. **76**. 15 p. 1927.
28. Johnson, J. The classification of certain virus diseases of the potato. Wisconsin Agr. Exp. Sta. Res. Bull. **87**. 24 p. 1929.
29. Kerling, L. C. P. The anatomy of the "Kroepoek-diseased" leaf of Nicotiana tabacum and of Zinnia elegans. Phytopath. **23**: 175–190. 1933.
30. Keur, J. Y. Studies of the occurrence and transmission of virus diseases in the genus Abutilon. Bull. Torrey Bot. Club **61**: 53–70. 1934.
31. Kirkpatrick, T. W. Further studies on leaf-curl of cotton in the Sudan. Bull. Ent. Res. **22**: 323–363. 1931.
32. Kunkel, L. O. A possible causative agent for the mosaic disease of corn. Bull. Exp. Sta. Hawaiian Sugar Planters' Assoc., Bot. Ser. **3**: 44–58. 1921.
33. Kunkel, L. O. Insect transmission of yellow stripe disease. Hawaiian Planters' Rec. **26**: 58–66. 1922.
34. Kunkel, L. O. Incubation period of aster yellows in its effect host. Phytopath. **16**: 67. 1926.
35. Kunkel, L. O. Studies on aster yellows. Am. Jour. Bot. **13**: 646–705. 1926.
36. Kunkel, L. O. The corn mosaic of Hawaii distinct from sugar cane mosaic. Phytopath. **17**: 41. 1927.
37. Kunkel, L. O. Celery yellows of California not identical with the aster yellows of New York. Contr. Boyce Thompson Inst. **4**: 405–414. 1932.

38. KUNKEL, L. O. Insect transmission of peach yellows. Contr. Boyce Thompson Inst. **5**: 19–28. 1933.
39. KUNKEL, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. **24**: 437–466. 1934.
40. LACKEY, C. F. Attenuation of curly-top virus by resistant sugar beets which are symptomless carriers. Phytopath. **19**: 975–977. 1929.
41. LACKEY, C. F. Further studies of the modification of sugar-beet curly-top virus by its various hosts. Phytopath. **19**: 1141. 1929.
42. LINFORD, M. B. Yellow-spot disease of pineapples transmitted by Thrips tabaci Lind. Science, n. s. **73**: 263. 1931.
43. LINFORD, M. B. Further studies of the transmission of the pineapple yellow-spot virus by Thrips tabaci. Phytopath. **21**: 999. 1931.
44. LINFORD, M. B. Transmission of the pineapple yellow-spot virus by Thrips tabaci. Phytopath. **22**: 301–324. 1932.
45. MAGEE, C. J. A new virus disease of bananas. Agr. Gaz. N. S. Wales **41**: 929. 1930.
46. MCKINNEY, H. H. Virus mixtures that may not be detected in young tobacco plants. Phytopath. **16**: 893. 1926.
47. MCKINNEY, H. H. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. Jour. Agr. Res. **39**: 557–578. 1929.
48. MCKINNEY, H. H. Étude sur les mélanges de virus. 2nd Cong. Intern. Path. Comp., Vol. **2**, Compt. Rend. et Commun. pp. 449–453. 1931.
49. MUNGOMERY, R. W., and BELL, A. F. Fiji disease of sugar-cane and its transmission Queensl. Bur. Sugar Exp. Sta. Div. Path. Bull. **4**. 28 p. 1933.
50. OCFEMIA, G. O. An insect vector of the Fiji disease of sugar cane. Am. Jour. Bot. **21**: 113–120. 1934.
51. OCFEMIA, G. O., and BUHAY, G. G. Bunchy-top of abacá or Manila hemp. II. Further studies on the transmission of the disease and a trial planting of abacá seedlings in a bunchy-top devastated field. Philipp. Agr. **22**: 567–781. 1934.
52. OGILVIE, L. A transmissible virus disease of the Easter lily. Ann. Appl. Biol. **15**: 540–562. 1928.
53. OSBORN, H. T. Incubation period of pea mosaic in the aphid, Macrosiphum pisi (Kaltenbach). Phytopath. **24**: ———. 1934.
54. PRICE, W. C. Isolation and study of some yellow strains of cucumber mosaic. Phytopath. **24**: 743–761. 1934.
55. PURDY, H. A. Immunologic reactions with tobacco mosaic virus. Jour. Exp. Med. **49**: 919–935. 1929.
56. QUANJER, H. M. The methods of classification of plant viruses and an attempt to classify and name potato viroses. Phytopath. **21**: 577–613. 1931.
57. RANKIN, W. H. Running-out of raspberries. New York Agr. Exp. Sta. Circ. **67**: 12 p. 1923.
58. RANKIN, W. H. Mosaic of raspberries. New York Sta. Agr. Exp. Sta. (Geneva) Bull. **543**. 60 p. 1927.

59. Salaman, R. N. Protective inoculation against a plant virus. Nature **131**: 468. 1933.
60. Samuel, G., Bald, J. G., and Pittman, H. A. Investigations on "spotted wilt" of tomatoes. Bull. Counc. Sci. and Ind. Res. Austr. **44**: 48 p. 1930.
61. Samuel, G., and Bald, J. G. Thrips tabaci as a vector of plant virus disease. Nature **128**: 494. 1931.
62. Schultz, E. S., and Folsom, D. Leafroll, net-necrosis, and spindling-sprout of the Irish potato. Jour. Agr. Res. **21**: 47–80. 1921.
63. Severin, H. H. P. Minimum incubation periods of causative agent of curly leaf in beet leafhopper and sugar beet. Phytopath. **11**: 424–429. 1921.
64. Smith, K. M. Studies on potato virus diseases. V. Insect transmission of potato leaf roll. Ann. Appl. Biol. **16**: 209–229. 1929.
65. Smith, K. M. Studies on potato virus diseases. IX. Some further experiments on the insect transmission of potato leaf-roll. Ann. Appl. Biol. **18**: 141–157. 1931.
66. Smith, K. M. Thrips tabaci Lind. as a vector of plant virus disease. Nature **127**: 852–853. 1931.
67. Smith, K. M. Studies on plant virus diseases. XI. Further experiments with a ringspot virus: its identification with spotted wilt of the tomato. Ann. Appl. Biol. **19**: 305–330. 1932.
68. Smith, K. M. Recent advances in the study of plant viruses. 423 p. J. and A. Churchill, London. 1933.
69. Smith, R. C., and Barker, H. D. Observations on the "yellows" disease of beans and related plants in Haiti. Jour. Econ. Ent. **23**: 842–847. 1930.
70. Smith, R. E., and Boncquet, P. A. Connection of a bacterial organism with the curly leaf of the sugar beet. Phytopath. **5**: 335–342. 1915.
71. Stahl, C. F. Corn stripe disease in Cuba not identical with can mosaic. Trop. Plant Res. Foundation Bull. **7**. 12 p. 1927.
72. Storey, H. H. The transmission of streak disease of maize by the leafhopper Balclutha mbila Naude. Ann. Appl. Biol. **12**: 422–439. 1925.
73. Storey, H. T. Transmission studies of maize streak disease. Ann. Appl. Biol. **15**: 1–25. 1928.
74. Storey, H. H., and Bottomley, A. M. Rosette disease of the peanut (Arachis hypogaea L.). Ann. Appl. Biol. **15**: 26–45. 1928.
75. Storey, H. H., and McClean, A. P. D. The transmission of streak disease between maize, sugar cane and wild grasses. Ann. Appl. Biol. **17**: 691–719. 1930.
76. Storey, H. H. The bearing of insect-vectors on the differentiation and classification of plant viruses. 2nd. Cong. Intern. Path. Comp., Vol. **2**, Compt. Rend. et Commun. pp. 471–477. 1931.
77. Storey, H. H. A new virus disease of the tobacco plant. Nature **128**: 187–188. 1931.

78. Storey, H. H. The inheritance by an insect vector of ability to transmit a plant virus. Proc. Roy. Soc., B. **112**: 46–60. 1932.
79. Storey, H. H. Leaf curl of tobacco in southern Rhodesia. Rhodesia Agr. Jour. **29**: 186–192. 1932.
80. Storey, H. H. Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. Proc. Roy. Soc., B. **113**: 463–485. 1933.
81. Stover, W. G., and Vermillion, M. T. Some experiments with a yellow mosaic of tomato. Phytopath. **23**: 34. 1933.
82. Summers, E. M. Types of mosaic on sugar cane in Louisiana. Phytopath. **24**: 1040–1042. 1934.
83. Takami, N. Stunt disease of rice and Nephotettix apicalis. Jour. Agr. Soc. Japan **241**: 22–30. 1901.
84. Thung, T. H. Smetstof en plantencel bij enkele virusziekten van de tabaksplant. Handeling. Nederl.-Ind. Natuurwetenschap. Cong. 6de **1931**: 450–463. 1931.
85. Thung, T. H. De krul- en kdoepoek-ziekten van tabak en de oorzaken van hare verbreiding. Proefsta. Vorstenl. Tabak, Meded. **72**. 54 p. 1932.
86. Venkata Rao, M. G., and Gopala Iyengar, K. Studies in spike disease of sandal. I. Two types of spike disease of sandal. Mysore Sandal Spike Investigation Committee Bull. **4**. 12 p. 1934.
87. Whitehead, T. Transmission of potato leaf-roll. Nature **125**: 974–975. 1930.
88. Department of Botany and Plant Pathology. Oregon Agr. Exp. Sta. Director's Bienn. Rept. **1926–28**: 78–82. 1930.

THE STRUCTURE OF PROTOPLASM

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The earliest work on protoplasm, done by such pioneers as Rösel von Rosenhof, Dujardin, von Mohl, and Purkinje was on living material. Then followed the work of the cytologists on fixed material. Much of interest and value resulted from these latter studies, notably the discovery of chromosomes and the phenomenon of mitosis. The new century ushered in a "new cytology" and with it not only a return to living material, but a new view-point based on modern physical and chemical interpretations. This change came about for a number of reasons. The now old cytology found itself, at least temporarily, in a *cul de sac;* the chemists were just beginning to offer their first substantial contributions to the study of colloidal systems, in particular, jellies; and the cytologist had come to realize that if he went no further than what he could see in his miscroscope, he would not get far, any more than would have the physicist had he stopped at what he could see and make a model of. Such methods, which dominated the mechanical interpretations of natural phenomena during the nineteenth century, had inevitabily to give way to interpretations based on behavior, whether the behavior of electrons, colloidal systems, or protoplasm. One can not rest content with visible structure.

The new cytology, from a strictly physiological point of view, may be said to have started with the work of Claude Bernard; from the chemical point of view, it began with the work of H. R. Proctor on "The Structure of Organic Jellies" (34).

Protoplasm was viewed by the older workers either as a fine granular suspension or as a framework of fibers, the latter forming a continuous net or an entanglement of discontinuous fibrils.

In 1894 Bütschli advanced his alveolar hypothesis of protoplasmic structure. He introduced the word *alveolus* (a small alveole or cavity) to indicate symmetrically arranged globules resembling small vacuoles and which, because of pressure, have assumed an angular form. Neither alveolus nor vacuole is, however, a well chosen term as there are no cavities or empty sacs in protoplasm. The alveolar structure is, in reality, that of an

emulsion, the globules of which are numerous, compact and under pressure; they, therefore, assume an angular and uniform shape and are symmetrically arranged, as in the case of the "cells" of a honeycomb.

There is no truth to the contention often made that the alveolar structure of protoplasm is always an artifact. It is clearly and perfectly presented in the ectoplasm of certain protozoa, e.g., *Euplotes,* but it is not of universal occurrence, nor of fundamental significance, as Bütschli maintained.

The emulsion hypothesis of protoplasmic structure is accepted by many (8, 9) though unfortunately not, in the author's opinion, wisely so. In its coarser structure, protoplasm, as seen through the microscope, is an emulsion, a dispersion of globules of varying size and distribution in an aqueous medium. When the globules are numerous, compact, angular and symmetrical, alveolar protoplasm results. When the globules are relatively large, uniform and spherical, they are termed *alveolar spheres* (55). When the globules appear to be "cavities" of the nature of small sacks, the structure is termed vacuolar (6). But it is impossible to differentiate clearly between a vacuole, a sack, a globule, an alveolus, or an alveolar sphere. All of these structures are but modifications of one and the same thing, namely, a globule; they all become, therefore, the dispersed phase of an emulsion.

One of the most interesting and significant among recent contributions to our knowledge of the protoplasmic emulsion is an observation by Spek (46, 48) who observed the fusion of several of the most minute of protoplasmic granules to form larger liquid globules with discernible contours. Thus does the "granular" structure fall under the heading of an emulsion. (Some solid granules occur in protoplasm, e.g., crystals). We, therefore, recognize one main type of visible structure in living protoplasm, namely, an emulsion. The various distinctive names which have been given to this structure indicate, in part, the actual particular configuration assumed by the emulsion and, in part, the author's interpretation of it as it exists in the particular material he has investigated.

The emulsion hypothesis.

There is no doubt that protoplasm, superficially viewed, is an emulsion. Doubt exists only as to the function and fundamental

nature of this emulsion. The emulsion hypothesis got a firm hold in biology for two reasons. First, protoplasm as seen through the microscope is quite evidently an emulsion and the colloidal structure of jellies was once thought to be a fine emulsion. The latter idea gave rise to the misleading word "emulsoid." It was very natural, therefore, for biologists to assume that the ultimate and hidden structure of protoplasm is an emulsion like the coarser and visible one, only finer. Let us first recall what happened to the emulsion hypothesis of the structure of jellies. Ellis (12) found that fine and pure emulsions are "model suspension colloids" and not of the jelly type at all. Hatschek (20), in a search for a possible mechanism in emulsions which would explain such gel properties as elasticity, analyzed the situation mathematically and concluded, "the theory that gels consist of two liquids must be pronounced untenable."

The first substantial contribution to the emulsion hypothesis of protoplasmic structure was that of Clowes (8) who evolved an ingenious theory of protoplasmic permeability. He assumed that the outer layer of protoplasm is an ultramicroscopic emulsion near the reversal point. When the emulsion swings slightly to one side or the other, toward the oil-in-water or water-in-oil state, it becomes more or less permeable to water soluble substances such as salts. The hypothesis nicely explains certain features of the permeability of protoplasm. Particularly convincing is Clowe's discovery that the proportion of sodium and calcium which keeps an emulsion at the reversal point is exactly that which exists in seawater, in blood and other physiological solutions.

A second substantial support to the emulsion hypothesis of protoplasmic structure, in reference to the surface layer, came from Dixon and Clark (10). They found that an electrical stimulus affects emulsions in the same way as it does protoplasm. An electric current will cause an emulsion, originally almost impermeable to ions and water soluble substances, to become fairly permeable, which is the same effect that electric stimuli have on living tissues, namely, they increase permeability. Dixon and Clark conclude, therefore, that an hypothesis which explains two such apparently unconnected and remarkable phenomena of the antagonistic action of ions on permeability, the hypothesis of Clowes, and the permeability changes produced by electric stimuli,

the work of Dixon and Clark, deserves serious consideration. This is true, yet it may simply mean that two rather diverse types of systems, an emulsion and a living jelly, show similar responses to the same environmental changes.

We are forced to discard the emulsion hypothesis of membrane control, in spite of two substantial facts in its support, that of Clowes and that of Dixon just cited, because of the following reasons. There is no direct evidence whatever of a phase reversal of the protoplasmic emulsion. Electrical conductivity measurements reveal that the conductivity of protoplasm is the same *at all viscosity values*. Blood also shows no change in conductivity in spite of a great increase in viscosity as a result of coagulation. This is true also of gelatin when it sets from a solution to a gel, and on this basis McBain (26) denied the possibility of phase reversal when soap jellies are formed. The whole idea of phase reversal has been discarded as a property of gel-forming systems of which protoplasm is one. It is very unlikely that protoplasm could exist as a living substance if fat were the continuous phase; metabolic reactions take place in aqueous media. As the stability of an emulsion increases with decrease in size of the dispersed particles, an ultramicroscopic emulsion will be extremely difficult to reverse, owing to a great increase in the surface tension of the stabilizing membrane. The amount of fat in the dispersed globules of an ultramicroscopic emulsion is probably insufficient to enclose the aqueous medium. There are, further, some very characteristic properties of protoplasm which cannot be explained on the basis of an emulsion structure. Protoplasm is elastic and emulsions, when pure, are not. Protoplasm coagulates and emulsions do not. When milk coagulates, it is a protein, caseinogen, which coagulates and not the emulsion of butter-fat.

The visible protoplasmic emulsion has its own important rôle to play. In addition to its nutritional properties, it presents a multitude of surfaces, and it is at surfaces chemical reactions take place. The microscopic protoplasmic emulsion may be present even when it is not visible with ordinary optical methods. That this is true is indicated by observations made with the Spierer lens (44, 49). This optical system is a Ziess 1/12″ f.l., 1.25 n.a. (.8 n.a. with iris closed), 90x, oil-immersion objective, on the lower lens of which a small metallic mirror has been placed which reflects all direct light,

thus permitting illumination of the material from below, as is customary in ordinary microscopic observations, yet giving a dark-field. The principle of the lens is based on the fact that the light scattered by a colloidal particle is most intense in the direction of the illuminating ray, according to the formula of Rayleigh (43). The Spierer objective is thus a complete dark-field system in itself, but it is used to advantage with a cardioid condenser. All structures seen with the Spierer lens in both protoplasm and cellulose have also been seen with cardioid and light-field optical systems, but less distinctly so. This statement is made because, as dark-field pictures owe their existence to diffraction phenomena, i.e., to the scattering of light, as do X-ray spectrograms, it is natural that question should arise in regard to the reality of structures revealed by dark-field, whether with the cardioid condenser or the Spierer objective. The first fact of significance is that no optical system can reveal structure of any type in a structureless space. The mere presence of diffraction phenomena is evidence of structure. While the structure revealed by diffraction phenomena may not be an exact counterpart of the actual structure, yet a linear orientation of parts in the picture must correspond to linear units in the object, while points indicate either a granular structure or a segmented linear one. A diffraction grating of 10,000 lines per inch viewed with ordinary light field and a 1/2″ oil immersion objective, and also viewed with the Spierer lens, shows the same number of lines per linear unit in both cases. There is no duplication by the Spierer lens. In the same manner, the Spierer lens changes nothing in the structure of protoplasm; it merely brings to light what is poorly seen by other methods. Obviously, diffraction lines, halos and like optical effects occur in all optical systems at times, particularly with dark-field illumination.

When apparently homogenous hyaline protoplasm is viewed through the Spierer lens it often presents the picture of an emulsion in which one, the dispersed, phase is brightly illuminated while the other, dispersion medium, remains dark. When the protoplasm is quiet the two substances present a mottled picture, a mosaic. The plant cell nucleus is of a similar mottled appearance; here the structure is often to be seen with ordinary direct illumination. When the protoplasm is under tension, as when formed into a thread, or when streaming, the emulsion assumes a striated appearance due

to a parallel arrangement of the illuminated and now elongated emulsion globules. Under stress, the globules becomes distorted into rods which are oriented end to end, sometimes so close as to appear to form a continuous thread. This structure, first brought out in detail and with strong contrast by the Spierer lens, had been previously revealed, less distinctly though definitely so, by ordinary light-field methods. A photograph by Scarth (36) shows the same structure in the streaming protoplasm of *Spirogyra*.

"Dispersed phase" and "dispersion medium" would be sufficient to designate the two parts of this emulsion, yet it seemed worth while (44), if for no other reason than to be certain that they will be definitely reckoned with, to give Greek names to the parts of this delicate visible protoplasmic emulsion. The brightly illuminated dispersed phase has been termed *phaneroplasm* (*phaneros* = evident), and the invisible, optically empty background or continuous phase, *cryptoplasm* (*cryptos* = hidden).

A number of other terms have been used which may possibly apply to phaneroplasm and cryptoplasm, though the authors of them seem to have reference to parts more comparable to the old idea of a "spongioplasm" (framework) and an "enchylema" (intervening substance). Examples of these are the terms coined by Strasburger, "kinoplasm" or active plasm and "trophoplasm" or nutritive plasm, terms which have been brought back into use by Lloyd & Scarth (23).

The more closely one approaches the ultimate structure of protoplasm, the less easy is it to differentiate vitally between the relative importance of its constituents, but if we attempt to distinguish between phaneroplasm and cryptoplasm from the view-point of their vital significance, then, discontinuity of the former and active streaming of the latter suggest that cryptoplasm, the continuous phase, is the more fundamental of the two.

The protoplasmic framework.

There has long persisted in the minds of biologists the thought that there must exist a continuous framework of some sort which is the structural background of protoplasm. Life in a dispersion (solution) of isolated units, no matter how complex the mixture, is inconceivable. Both this theoretical concept and actual observations on fixed and stained material indicated the presence in proto-

plasm of a structure, variously described, but in all instances consisting of a meshwork or entanglement of fibers, forming a three-dimensional net or sponge. The idea of continuity in protoplasmic structure is thoroughly sound and is supported by ample evidence, but much, though by no means all, of the cytological support, based on fixed material, given to it, is faulty. The *fibrillar* hypothesis, advanced by Flemming and others, ascribes to protoplasm the structure of an entanglement of fibrils. Flemming elevated these fibrillae, as did Altmann his granules, above the lowly station of mere structural units and viewed them as the seat of the energies on which life depends. The drawings of Flemming of connective tissue, of Heidenhain of muscle and spinal ganglion cells, and preparations of Strasburger (41) depict a fibrillar structure. Such a structure is characteristic of and visible in certain living tissues. Ettisch (13), with the aid of dark-field illumination, finds the construction of sinew to be that of an aggregation of minute fibers.

The fibers so far referred to are of microscopic dimensions but are built up of finer ultramicroscopic, invisible fibers, probably present in living tissue generally.

Linear structural units may be oriented so as to form an entanglement such as exists in a brush-heap, or they may be arranged in a more orderly manner in the fashion of a three-dimensional net. Earlier controversies often centered on the question whether protoplasmic fibers are discontinuous or whether they anastomose to form a *reticulum*. The meshes of the supposed protoplasmic net were said to be from 1/2 to 2 μ in size. Whether the purely anatomical framework or the hyaloplasm, "enchylema," which bathes it, is the real living substance, was judged in the favor of the latter.

The concept of a reticulum as the structural framework of protoplasm has persisted in medicine in the widely recognized *stroma* in the red blood cell. The stroma is presumed to be a delicate web-like net, peripherally located. Microdissection studies fail to reveal any such framework either in the large (nucleated) amphibian erythrocyte or in the human corpuscle (39). While the concept of a continuous framework is a justifiable one and finds ample evidence in other material, the red blood cell is simply a sack.

The reticular, fibrillar, net and sponge-like structures seen in fixed protoplasm may be true fibrous coagula or pictures of an emulsion caught in a coagulum; for example, "chromatin granules" on a "linin thread," a structure which has played a prominent rôle in modern cytological and genetical theories of nuclear behavior, is readily produced by a distorted emulsion of large globules with a minimum of dispersion medium (42). The "granules" would then be the points where several globules approach each other and the "linin thread" would be the connecting strands of the dispersion medium. This is shown by comparing a typical drawing of chromatin material, showing "chromatin granules" on a "linin thread," with one or more of the possible configurations of an emulsion. The symmetrical arrangement of the phases of an emulsion might well pass for the picture of a net or reticulum.

With the older concept of a framework as the structural basis of protoplasm in mind but with the realization that the earlier evidence for it is not always sound, let us turn to modern theories.

The ultramicroscopic structure of protoplasm.

The ultramicroscopic structure of protoplasm, like that of non-living matter, is obviously not visible, but theories pertaining to it, as to molecular and atomic structure, are based on sound though indirect evidence. We can best approach our problem by a simple analogy. Of two soap solutions, one of low concentration and low viscosity, and one of high concentration and high viscosity, the former was elastic and the latter not; the former held a small metal particle in suspension, while the latter could not support the same particle. It would seem, therefore, that the elastic yet thin soap solution possessed a structure which would account for its elastic qualities and for its ability to support a metal particle, while the thicker yet inelastic soap lacked such a structure. This supposition was supported by microscopic examination. The elastic soap solution contained long and slender crystals, while the other soap resembled chalk dust. We have in the behavior and structure of these two soaps the basis of all generally accepted hypothesis of the structure of jellies. Elastic colloidal systems are built up of linear crystalline units. Their intermeshing gives elasticity and rigidity to liquids which yet flow freely and smoothly. This is structurally possible if we regard the framework of fibers as not

fixed but labile, capable of readjustment and comparable to a loosely put-together brush-heap. A brush-heap is elastic; a sand pile is inelastic.

Before carrying the story of the fibrous structure of protoplasm over to cellulose, investigations on which have yielded much in regard to gel structure in general, let us see how the intermeshed fibrous structure is associated with the protoplasmic emulsion. Milk illustrates the situation almost perfectly. Viewed through the microscope, milk is an emulsion of butter-fat in an aqueous medium. More than this is not visible. When milk coagulates the emulsion plays only a passive part. It is the casein in milk which coagulates. The fluid whey, an aqueous solution of salts, sugars, etc., separates from the casein coagulum. There are thus in milk three quite distinct systems, intimately associated, namely, an emulsion of fat, a dispersion of fibrous units capable of forming a coagulum, and a solution, of salts, etc., permeating the whole. So it is with protoplasm.

Investigations on the structure of cellulose give the best possible insight into modern interpretations of the mechanism underlying the behavior of colloidal jellies, including protoplasm.

The structure of cellulose.

The cellulose molecule is now thought to be a chain built of rings of anhydrous glucose ($C_6H_{10}O_5$) (28, 50, 51). This latter group has long been known to be the basic unit of cellulose and all higher carbohydrates, but the number and arrangement of the rings in the larger cellulose molecules were not known. It is now believed that in cellulose each ring is joined to its neighbor by an oxygen bridge, and every alternate ring is the reflected image of the one on each side of it, i.e., it is rotated through 180°. Two such rings constitute an anhydrous molecule of the sugar cellobiose ($C_{12}H_{22}O_{11}$). Some forty or more of these rings, so-called glucose "residues," joined in a continuous chain, form the cellulose molecule. The length of the chain is not fixed. It is capable, stoichiometrically at least, of reaching any length. One can not, therefore, speak of a cellulose molecule in the strict sense if by molecule is meant a unit of fixed weight and constitution. A length of forty glucose residues, or twenty times the length of the cellobiose molecule (10.3 A. U.), represents a chain length of about 200 A. U. This is a

minimum. Two or three times this probably more closely represents an average. The "macro-molecule" of the cotton fiber appears to be the longest, 1000 A. U. Physically, the molecules must be regarded as comparatively stiff threads.

The linear cellulose molecule has at each end an apparently unsatisfied valence bond. There is little likelihood that such a free carbon bond actually exists; it rather indicates where our knowledge ends. The bond is possibly satisfied by a univalent (OH) group or joined to an adjoining chain.

The molecular weight of this long chain molecule is now put at 30,000 to 40,000. Stamm (52) obtained the latter value by centrifuging in a high speed Svedberg centrifuge. As the length of the chain varies, the molecular weight will vary.

There are many polymeric materials which are constituted on the same principle as cellulose in that their molecules are characterized by a chain of recruiting structural units; rubber is such a substance.

With this information as a starting point—though it was at the time less precise than now—the problem was carried forward by the X-ray workers (25, 29, 51). The spectrograms obtained indicate clearly that the structure of cellulose is symmetrical, that is to say, crystalline.

The long cellulose chains are aggregated into bundles of some sixty chains each. These bundles, being molecular aggregates, satisfy Nägeli's definition of a *micelle*. We shall recall that the botanist Nägeli postulated a so-called micellar structure for all gels, including protoplasm, the unit of the structure being a micelle or aggregate of molecules, i.e., a colloidal particle. As the cellulose micelle is symmetrical in structure and, therefore, crystalline, it has received the name of *crystallite*. An association of cellulose crystallites, oriented much as are bricks in a wall, presumably constitutes the colloidal structure of cellulose (45).

The precise orientation of the micelles is of significance in such properties as electric conductance, tensile strength, and elasticity. Mark (25) depicts two extremes, one in which there is perfect parallelism, and one in which there is a random or brush-heap distribution of the micelles, the former represented by native ramie and the latter by cellophane. The cellulose of flax displays an

excellent orientation of micelles parallel to the fiber axis, and has a tensile strength comparable to the best steel.

Carothers (5) adds another possibility, namely, that of pronounced overlapping of the molecules of one bundle with those of another, a very likely condition in that the molecular chains of a cellulose micelle are of different lengths. Such an arrangement would provide maximum strength in the direction of the fiber axis, because the mutual cohesive force of the long chains would be fully utilized. In regeneration cellulose (cellophane), says Carothers, there is random orientation. The molecules are brought into an ordered arrangement by mechanical stress. The strength of a sheet of cellophane which is initially the same in all directions, can be so changed by stretching that its strength along the axis of stretch is increased several times.

If we turn for a moment to other substances of an organic nature which have been subjected to X-ray study and found to be crystalline in nature, with linear units in often orderly arrangement, we find that the list is a long one; it includes starch, gelatin, chitin, rubber, silk, hair, keratin, sinew, muscle, nerve, and brain. It is but a step from these to protoplasm; indeed, muscle, nerve, and brain are protoplasm.

Frey-Wyssling (17, 18) has carried out extensive polarization studies on the cell walls of plants. The method is that of immersion in liquids of known index of refraction, and observation through Nicol prisms. By this means he showed that in cell walls there are submicroscopical (colloidal) rod-shaped particles which he identifies with the Nägeli micelles. The long axis of each micelle corresponds to the direction of the greatest refractive index; the latter value, therefore, gives the orientation of the micelles in the wall.

Photographs of cellulose taken with the Spierer lens add further evidence to the general conclusion that cellulose possesses a colloidal structure of symmetrically arranged rods (43). The lens reveals parallel striae which appear to be composed of microscopic units or *super-micelles,* oriented end to end. The striae form lamellae or plates which in their turn combine to produce the mass of cellulose. The same striated and articulate structure persists in bituminous coal as shown by Thiessen (53).

Question has arisen over the superficially similar structure shown by the Spierer lens in protoplasm and in cellulose. In both cases the structure is that of short rods, linearly oriented in parallel striae. That this confusion should arise is understandable, but the resemblance is purely superficial. If rolling country adjoining the sea, where large waves for the moment exist, is viewed from a distance, both land and sea would present the picture of parallel ridges, yet the material of which they are made and the forces responsible for their existence are entirely different in the two cases. So it is with protoplasm and cellulose in their finer microscopic structure. The punctated striae in the case of cellulose are built of oriented short rods, or super-micelles, of solid material. The punctated striae in the case of protoplasm are built of distorted, rod-shaped, liquid droplets, the dispersed phase of an emulsion under tension.

The exceedingly delicate macroscopic fibers of which wood is composed are built ultramicroscopic and molecular fibrils, such as those to which we have referred. The wood fibers in their turn build up the larger fibers characteristic of plan cellulose, e.g., cotton fibers. Natural cellulose thus consists of units of ever increasing size, all of which, from chain molecules to visible wood fibers, are of linear form. The orientation of these units determines the physical properties (elasticity, tensile strength, etc.) of the material.

Probably no other force in nature is so widely distributed and plays so great a rôle in the behavior of systems, from molecules to organisms, as does polarity. The term expresses any situation where the two ends or sides of an object are different, but in the strict chemical sense polarity should be limited to objects the ends of which are electrically unsymmetrical. Given long and polar molecules, molecules with ends electrically dissimilar, it is possible to picture their orientation in mass and to obtain a type of structure which is presumably typical of gels, and which at least has the virtue of giving a mechanical basis upon which to interpret the behavior of gels. Any linear molecule with unsatisfied terminal or lateral bonds, such as amino acids with ionized NH_3^+ and COO^- groups or protein molecules with side chains, presents the possibilities of weak unions along the main chain. So-called internal salt

formation in proteins, and the many examples of tautomeric shifts, are similar cases. Such a situation meets the structural requirements of a brush-heap of loose construction, capable of constant readjustment. It is well illustrated in gels which exhibit the phenomenon known as thixotropy (15) which is a very typical property of protoplasm. The term refers to the sudden collapse of a gel from a firm body to a thin fluid as a result of mere mechanical disturbance. In ideal thixotropic systems, ferric oxide sol, bentonite, etc., the gel is reformed, again and again. Possibly all sudden changes in protoplasmic consistency, if not also muscular action, are instances of thixotropic change.

Viscosity measurements of protoplasm (40) have played a prominent part in protoplasmic structure, not so much because of the values obtained, but rather because of the discrepancy in values. Among the reasons for this discrepancy is the non-Newtonian (anomalous) behavior of protoplasm. It does not exhibit true viscous flow. This is denied by some workers who believe protoplasm to be a true solution with no "yield value" such as is characteristic of colloidal lyophilic solutions. Other reasons which may explain the divergence in values of protoplasmic consistency are methods in measuring, failure to realize that protoplasm undergoes very rapid changes in viscosity, and that, with possible rare exceptions, all parts of the cell are not of the same consistency.

Many workers have studied protoplasmic consistency because of its important bearing on physiological reactions, such as protoplasmic streaming, amoeboid movement, metabolic activity and muscular contraction. Where the change in viscosity is exceedingly rapid, thixotropy, rather than a simple viscosity change, is probably responsible.

Pure liquids, e.g. glycerine, and pure solutions have one viscosity value at all pressures. Lyophilic colloidal solutions, e.g. a gelatine sol, have different viscosity values at every pressure at which they are measured. They are said, therefore, to be non-Newtonian, because Newton's law of viscous flow does not apply. The fact that they deviate from this and Poiseuille's laws, indicates that there are structural features which interfere with pure viscous flow. The non-Newtonian or anomalous behavior of colloidal solutions is one of the best indicators we have of a continuity in structure. All such substances, proteins, etc., possess a *yield value,* i.e., they re-

quire the application of a force to start flow. If they lack a yield value they are Newtonian and show true viscous flow. The single constant laws of Newton, Maxwell, and Hooke do not apply rigidly to non-Newtonian systems nor do the laws of Stoke and Poiseuille. As protoplasm is elastic, exhibits thixotropic behavior, and contains a high proportion of protein, it is inconceivable that it should show true viscous flow. It will approach this latter condition when thin.

A number of apparently inconsistent results pertaining to protoplasmic consistency are capable of interpretation on a structural basis. One worker, by observing the Brownian movement of particles, obtains a low value for the viscosity of protoplasm. The particles may be in minute vacuoles (24) and the values, therefore, applicable only to the fluid aqueous medium within and not to the protoplasmic mass as a whole. Osmotic measurements, which suggest that protoplasm is a true solution, may apply only to the aqueous dispersion medium which bathes the protoplasmic framework. Such observations tell nothing of the structural features of protoplasm as an entity, of those features which are necessary to account for thixotropic behavior, elastic qualities, and immiscibility in water. Protoplasm *imbibes,* i.e., takes up, water; it does not *dissolve* in water; this implies structural continuity. Such properties of protoplasm are the best criteria we have of protoplasmic structure.

Scarth (36) says that protoplasm is characteristically elastic and the impression of fluidity is illusory. He cites the case of active streaming in freely suspended protoplasmic strands, which is possible only if there is a structural framework. Spek (46) is of the same opinion. So apparently is also E. B. Wilson (55) when he states that the "continuous substance" is the most constant and active element of protoplasm and forms the structural basis of the system. E. G. Conklin (9) adds that protoplasm is composed of a more fluid and a more viscid portion. He bases his statement on experiments in centrifuging the eggs of *Crepidula* where he found that the more fluid portion of protoplasm may be readily moved but the more viscid portion is not so readily moved; the more viscid part of the protoplasm holds the nucleus in definite relation to the periphery of the cell and brings parts back to their normal positions when once they have been displaced by centrifuging.

Another property which is typical of elastic jellies is that of *syneresis,* or the squeezing out of some of the aqueous medium by a slow contraction of the gel. The property is nicely exhibited in protoplasm, being most pronounced when abnormal conditions arise.

If we now list those properties of protoplasm which force us to recognize it to be essentially a jelly, a lyophilic colloidal system, whether firm or fluid, they are: elasticity, rigidity, extensibility, imbibition, water-immiscibility, thixotropy, syneresis, and coagulation.

One of the obstacles to the universal acceptance of structural continuity in protoplasm has been the notion that a framework is inconsistent with the evident fact that protoplasm flows. So do thixotropic solutions flow, but they immediately build up again into firm gels. The framework of protoplasm is constantly changing. The linear structural units undergo continuous readjustment, due possibly to tautomeric shifts.

It is significant for the problem of protoplasmic structure to realize that while polarization studies of protoplasm have not shown living matter generally to be anisotropic yet they have shown striated muscle, types of connective tissue generally, and chlorophyll to be anisotropic. Furthermore, muscle, nerve, and brain, which are protoplasm, have yielded spectrograms (X-ray diffraction patterns) as have sinew, hair, silk, etc., indicative of a crystalline nature.

Support for linear units in protoplasm comes from a considerable diversity of observations. Fibrous structures are typical of fixed cells. Living protoplasm is often of a "stringy" appearance and is of high tensile strength. Protoplasmic strands may snap with great suddenness and recoil. Muscle is fibrous (13). Nerve tissue is a bundle of threads. A. R. Moore (30) finds that plasmodia when forced through moderately fine sieves do not live, but they may of themselves flow through exceedingly fine sieves. Forcing crushes the long protoplasmic fibers, while in flowing naturally the protoplasm can take the fibers through very fine pores. Moore believes the microfibrils to be of the order of 5×10^{-5} mm. in diameter and 2,000 times as long. Peters (33) has postulated similar but finer molecular threads in protoplasm. Needham (32) discusses the importance of the problem.

The possible significance of cytoplasmic structure in physiological behavior is indicated by A. R. Moore (31) who finds that neither sperm nor egg nucleus of echinoderms has any effect on segmentation tempo, the reactions of the cytoplasm alone determining it.

The eternal question of how it is possible for protoplasm to carry on so many different processes simultaneously, without one interfering with the other, within the confines of a single cell, may be answered by the justifiable supposition that delicate membranes, consisting of nothing more than firm protoplasm, traverse the cell in all directions. An excellent example of this is to be had in a myxomycete plasmodium where there are set up temporary channels or arteries of protoplasmic flow. These arteries guide the protoplasm along definite routes which are broken down and reestablished as the plasmodium progresses. The streaming protoplasm does not pass beyond the ephemeral boundaries of the arteries, though the latter are also of protoplasm. Their formation and temporary maintenance are undoubtedly made possible by a structural (fibrous) framework which endows the membranes with the required degree of rigidity.

Protoplasmic organization.

Whatever life may be and however much we may try to explain it on the basis of relatively simple phenomena, there always remains that greatest of all bodily and protoplasmic qualities, *organization.* To fully interpret cellular or protoplasmic organization in physical terms is, in the present state of our knowledge, impossible. The living system is too intricate; it is life itself. We believe, however, that the most fundamental characteristic of organization is structure. A study of chemical constitution alone will go no further in revealing the mechanism of even the simplest processes in protoplasm than it has in non-living systems. Structure, as well as chemical constitution, and the dynamics resulting from both, are necessary. The structure responsible for protoplasmic organization and most other physical properties of protoplasm, is a continuous but labile framework. Life in a discontinuous system is inconceivable. Aggregation, not dispersion, is the rule in living, as it is in non-living, colloidal phenomena. The harmonious functioning of a cell, which is but another name for life, is possible only because of the structural continuity of protoplasm.

Bibliography

1. Bělařr, K. Mechanismus der indirekten Kernteilung. Naturwiss. **15**: 725. 1927.
2. ————. Kausalanalyse der Mitose. Zeitsch. Zellforsch. u. mikrosk. Anat. **10**: 73. 1929.
3. ————. Beiträge zur Kausalanalyse der Mitose. Arch. Entwicklungsmech. Organismen **118**: 359. 1929.
4. Bogue, R. H. The chemistry and technology of gelatin and glue. New York, 1922.
5. Carothers, W. H. Polymerization. Chem. Rev. **8**: 353. 1931.
6. Chamberlain, C. J. Comparative morphology of cytoplasm and chromatin. Bot. Gaz. **80**: 203. 1925.
7. Chambers, R. The physical structure of protoplasm, V. General Cytology. (Cowdry), Chicago, 1924.
8. Clowes, G. H. A. Protoplasmic equilibrium. Journ. Phys. Chem. **20**: 407. 1916.
9. Conklin, E. G. Effect of centrifugal force on the structure and development of the eggs of *Crepidula*. Journ. Exp. Zool. **22**: 311. 1917.
10. Dixon, H. H., and Bennet-Clark, T. A. Electrical properties of oil-water emulsions, with special reference to the structure of the plasmatic membrane.—II. Sci. Proc. Royal Dublin Soc. **20**: 211. 1932.
11. Dufrenoy, J. Death as the result of change of living matter. Science **78**: 494. 1933.
12. Ellis, R. A neutral oil emulsion as a model of a suspension colloid. Trans. Farad. Soc. **9**: 14. 1913.
13. Ettisch, G., and Szegvari, A. Der Feinbau der kollagenen Bindegewebsfibrille. Protoplasma **1**: 214. 1926.
14. Freundlich, H. Neuere Fortschritte der Kolloidchemie und ihre Biologische Bedeutung. Protoplasma **2**: 278. 1927.
15. Freundlich, H., Enslin, O., and Söllner, K. Bildung von Taktoiden und ihre Biologische Bedeutung. Protoplasma **17**: 489. 1933.
16. Frey, A. Mikroskopische Technik der Micellaruntersuchungen von Zellmembranen. Zeitsch. wiss. Mikrosk. **47**: 1. 1930.
17. Frey-Wyssling, A. Die Stoffausscheidung der höheren Pflanzen. Leipzig, 1935.
18. Fry, Henry J., and Parks, Mark E. Studies of the mitotic figure. IV. Mitotic changes and viscosity changes in eggs of *Arbacia*, Cumingia, and Nereis. Protoplasma **21**: 473. 1934.
19. Greenberg, D. M., and Mackey, M. A. The sol-gel transformation in gelatin. Journ. Gen. Physiol. **15**: 16. 1931.
20. Hatschek, E. An analysis of the theory of gels as systems of two liquid phases. Trans. Farad. Soc. **12**: 17. 1917.
21. Leathes, J. B. Function and design. Science **64**: 387. 1926.

22. Laing, M. E., and McBain, J. W., Sodium oleate solution in three physical states. Journ. Chem. Soc. **117**: 1506. 1920.
23. Lloyd, F. E., and Scarth, G. W. The rôle of kinoplasm in the genesis of vacuoles. Science **65**: 599. 1927.
24. Mast, S. O. Structure, movement, locomotion and stimulation in *Amoeba*. Journ. Morph. & Physiol. **41**: 347. 1926.
25. Mark, H. Der Aufbau der hochpolymeren organischen. Naturstoffe. Leipzig, 1930.
26. McBain, J. W. Colloid chemistry of soap. Brit. Assoc. Advanc. Sci., Third Rep. Colloid Chem. **2**: 1920.
27. ————. A survey of the main principles of colloid science. Colloid Symposium Mono. **4**: 7. New York, 1926.
28. Meyer, K. H. Neue Wege in der organischen Strukturlehre und in der Erforschung hochpolymerer Verbindungen. Naturwiss. **16**: 781. 1928.
29. ————. Die Chemie der Micelle und ihre Anwendung auf biochemische und biologische Probleme. Biochem. Zeitsch. **208**: 1. 1929.
30. Moore, A. R. On the cytoplasmic framework of the plasmodium, *Physarum polycephalum*. Sci. Reports Tohoku Imp. Univ. **8**: 189. 1933.
31. ————. Is cleavage rate a function of the cytoplasm or of the nucleus? Journ. Exp. Biol. **10**: 230. 1933.
32. Needham, J. On the dissociability of fundamental processes in ontogenesis. Biol. Rev. **8**: 180. 1933.
33. Peters, R. A. Surface structure in the integration of cell activities. Trans. Faraday Soc. **26**: 797. 1930.
34. Proctor, H. R. The structure of organic jellies. Proc. Seventh Internat. Cong. App. Chem. London, 1909.
35. Runnström, J. Veränderung der Plasmakolloide bei der Entwicklungserregung des Seeigeleies. Protoplasma **5**: 201. 1929.
36. Scarth, G. W. The structural organization of plant protoplasm. Protoplasma **2**: 189. 1927.
37. Schmidt, W. J. Die Bausteine des Tierkörpers in Polarisierten Lichte. Bonn, 1924.
38. ————. Naegelischen Michellarlehre bei der Erforschung des Organismus. Naturwiss. **16**: 900. 1928.
39. Seifriz, W. The physical properties of erythrocytes. Protoplasma **1**: 345. 1926.
40. ————. The viscosity of protoplasm: molecular physics in relation to biology. Bull. Nat. Res. Council **69**: 229. 1929.
41. ————. The contractility of protoplasm. Amer. Naturalist **63**: 410. 1929.
42. ————. The alveolar structure of protoplasm. Protoplasma **9**: 177. 1930.
43. ————. The Spierer lens and what it reveals in cellulose and protoplasm. Journ. Phys. Chem. **35**: 118. 1931.

44. ————. The structure of protoplasm. Science **73**: 1. 1931.
45. ————. The origin, composition and structure of cellulose in the living plant. Protoplasma **21**: 129. 1934.
46. SPEK, J. Zum Problem der Plasmastrukturen. Zeitsch. Zellen und Gewebelehre. **1**: 278. 1924.
47. ————. Gesetzmässige Substanzverteilung bei der Fuerhung des Ctenophoreneies. Arch. Entwicklungsmech. Organismen **107**: 54. 1926.
48. ————. Plasmakolloide bei Befruchtung und Entwicklung des Nerieseies. Protoplasma **9**: 370. 1930.
49. SPIERER, C. Un nouvel ultra-microscope. Arch. Sci. phys. et natur. **8**: 121. 1926.
50. SPONSLER, O. L. The molecular structure of the cell wall of fibers. A summary of X-ray investigations. Amer. Journ. Bot. **15**: 525. 1928.
51. SPONSLER, O. L., AND DORE, W. H. The structure of ramie cellulose as derived from X-ray data. Colloid Symposium Mono. **4**: 174. 1926.
52. STAMM, A. J. The state of dispersion of cellulose. Journ. Amer. Chem. Soc. **52**: 3047. 1930.
53. THIESSEN, R. Physical structure of coal, cellulose fiber and wood as shown by the Spierer lens. Ind. Eng. Chem. **24**: 1032. 1932.
54. WILSON, J. A. Swelling of protein jellies. Colloid Symposium Mono. **1**: 210. 1923.
55. WILSON, E. B. The Cell. New York, 1925.
56. ZOCHER, H. Freiwillige Struckturbildung in Solen. Zeitsch. Anorg. Chem. **147**: 91. 1925.

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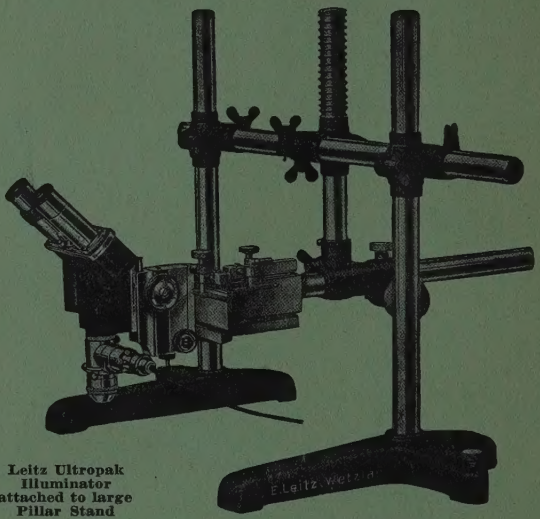

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